0073-5655/81/0926-0934 $01.50/0
IN VITRO Vol. 17, No. 10, October 1981

MOUSE LIVER CELL CULTURE

II. Primary Culture

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(Received December 8, 1980; accepted February 16, 1981)

SUMMARY

Mouse hepatocytes in primary culture were characterized. Hepatocytes were isolated by the two-step hepatic portal vein perfusion method described previously. An optimal cell attachment of 43% was noted after 2 h incubation in 10% fetal bovine serum. Minimal attachment (less than 7%) occurred in serumless medium. Serum concentrations above 10% and attachment durations greater than 2 h resulted in no increased attachment of viable cells. Nonviable cells, however, progressively attached when both of these parameters were increased. Survival data of the cells in culture resembled those reported for rat hepatocytes in primary culture. A progressive decrease in survival was noted following initial attachment until only approximately 15% of initially plated cells remained viable and attached after 8 d culture. The decrease in survival was accompanied by morphologic changes including flattening and elongation of the cells, some multinucleation, and disruption of monolayer groups.

Key words: mouse hepatocytes; primary culture; attachment.

INTRODUCTION

Several laboratories have been successful in the primary culture of adult rat hepatocytes (1-6). Biochemical (7-9) and morphological (2,3,10) characteristics of these cells during culture have been investigated. Both attachment efficiency and survival of hepatocytes during culture have been shown to be influenced by culture conditions. The percentage of serum, duration of incubation, and the number of viable cells plated have been shown to be important factors influencing the attachment efficiency (2,3,6,7). Cell survival during culture appears to be influenced by the medium used (6), the addition of various hormones in the medium (3,11,12), and the substrate on which the cells are grown (5).

Despite the number of studies that have been concerned with the primary culture of rat hepatocytes, little effort has been directed toward the primary culture of hepatocytes from other mammalian species. The mouse liver, in particular, would seem to be an excellent source of cells for culture, inasmuch as the mouse and the rat have been the principal models in vivo for carcinogenicity and toxicity studies, and primary rat liver cell cultures have been shown to be of value in in vitro toxicity (8,13) and carcinogenicity (14-19) investigations.

Renton et al. (19) recently reported the isolation and short-term primary culture of mouse hepatocytes on floating collagen membranes. However, quantitative data on the behavior of these cells in culture were not reported.

The present report described a quantitative investigation into the attachment, survival, and morphologic characteristics of mouse hepatocytes in primary culture. The influence of serum concentration, type of medium used, and duration of attachment were compared for the production of optimal attachment efficiency. The number of viable and nonviable hepatocytes that remained attached during the first 8 d culture were quantitatively determined. Phase contrast microscopy of cells in culture also was performed.

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MATERIALS AND METHODS

Isolation. Liver cells were isolated from adult male BALB/c mice (30 to 35 g each) using the two-step hepatic portal vein perfusion technique described previously (20). Hanks' calcium- and magnesium-free salt solution (containing 0.5 m*M* ethylene glycol-bis-(β-amino ethyl) *N,N'*-tetracetic acid (EGTA) and 0.05 *M* HEPES) and collagenase (100 U/ml) in Leibovitz L15 medium were used as the perfusates. Only cells from isolations in which hepatocyte viability was greater than 90% were used in subsequent cell attachment and cell survival experiments.

Cell attachment. The influence of serum concentration and duration of incubation on cell attachment efficiency was assessed. Isolated hepatocytes from one mouse were plated on 25 cm^2 tissue culture flasks (Falcon Plastics, Los Angeles, CA) at a concentration of 1×10^6 viable cells per flask in 5 ml L15 medium containing 50 μg/ml of gentamicin and either 0, 5, 10, 15, or 20% heat inactivated fetal bovine serum (Grand Island Biological Co., Grand Island, NY). Flasks, with their caps loosened, were maintained in a humidified 100% air incubator at 36.5° C.

The number of attached viable cells was determined at 1, 2, 3, and 4 h after plating using an in situ counting procedure similar to that of Martinez-Lopez and Black (21) as modified by Williams et al. (6) for rat liver cells. This involved first washing the flasks for 10 min with serumfree L15 medium to remove unattached cells followed by flooding the flasks with 5 ml serumfree L15 medium containing 0.2 ml 0.4% trypan blue dye for 5 min at 36.5° C. After treatment, the trypan blue dye containing medium was removed and attached; viable trypan blue excluding cells and nonviable trypan blue staining cells were counted in 10 0.25 mm^2 reticle fields per 25 cm^2 flask observed under an inverted phase contrast microscope at 100× magnification. Three flasks per experiment were counted for each serum concentration at each time period. A total of 10 experiments (10 mice) were conducted in 30 flasks counted per variable.

The type of antibiotic used in the culture medium has been reported to influence cell attachment. Waymouth and Ward (22) noted a decrease in attachment efficiency of newborn mouse liver cells when using penicillin/streptomycin in the medium. In contrast, gentamicin in that study had no influence on liver cell attachment. To evaluate the possible effect of antibiotics on adult mouse liver cell attachment, five formulations of complete L15 medium (with 10% heat inactivated fetal bovine serum) containing either: (a) gentamicin (50 μg/ml); (b) gentamicin (50 μg/ml) and amphotericin (0.25 μg/mg); (c) penicillin (100 U/ml) and streptomycin (100 μg/ml); (d) penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin (0.25 μg/ml); or (e) no antibiotics were compared for influence on cell attachment. Isolated liver cells from a single mouse were plated at a concentration of 1×10^6 viable cells per 25 cm^2 tissue culture flask and attachment was assessed by the in situ counting procedure described above.

Cell survival. Liver cell survival during the first 8 d culture was also quantitatively determined. Isolated cells were plated onto 25 cm^2 tissue culture flasks at a concentration of 1×10^6 viable cells per flask. Five milliliters of either Williams' WE medium plus 10% heat-inactivated fetal bovine serum or L15 medium plus 10% heat-inactivated fetal bovine serum were added to each flask. All flasks were incubated at 36.5° C. Flasks containing WE medium were maintained in a humidified 95% air:5% CO_2 incubator.

Medium was changed after optimal attachment (*see* Results), after the first 24 h, and each 48 h thereafter. The number of attached nonviable and viable cells were counted in situ using the method outlined above at Day 1, 2, 4, 6, and 8. Triplicate flasks were counted for each time period per experiment. A total of five experiments were conducted. Each experiment represented the isolated cells from one mouse. Liver cell morphology was observed and photographed with a phase contrast inverted microscope during the first 8 d of culture.

RESULTS

Attachment. The effect of serum concentration and duration of incubation on liver cell attachment is shown in Table 1. With no serum addition to the medium, little liver cell attachment was observed over the 4 h incubation. A maximum attachment efficiency of 7% occurred with serumless medium at 3 h incubation. The inclusion of 5% serum in the medium increased the percentage of attached viable cells to a maximum of approximately 34%, which was seen 2 h after plating. The attachment of viable cells in 5% serum remained constant at this percentage even after the 3rd and 4th h. No significant difference was noted in the number of attached viable liver cells when 10, 15, or 20% serum was included in the

medium. Maximum attachment in these latter three serum concentrations occurred after 2 h incubation, and no increase was noted there after 2 h. Two hours, therefore, for all serum concentrations tested (except serumless medium) provided maximum attachment of viable cells in the shortest time tested after plating.

Time after plating also influenced the number of attached nonviable (trypan blue staining) liver cells (Table 1). For all serum concentrations studied, the percentage of nonviable cells attached to the flasks increased with time.

Increasing the serum concentration above 10% and increasing the time after plating beyond 2 h resulted in no significant increase in the percentage of viable cells that attached. Therefore, a serum concentration of 10% heat inactivated fetal bovine serum and a duration of 2 h following the initial plating were used to obtain optimal cell attachment in subsequent studies. Although L15 medium was used throughout the above experiments, WE medium produced a similar pattern of attachment. No significant difference was noted in the attachment of either viable or nonviable liver cells with any of the antibiotic combinations used (data not shown).

Cell survival. Survival data of hepatocytes during the first 8 d culture are shown in Table 2. After attachment, hepatocytes displayed a constant, steady decline of survival. After 8 d, fewer than 15% of the initially plated cells remained viable. Similarly, the percentage of trypan blue staining (nonviable) cells showed an increase with time in culture apparently reflecting the degeneration of initially attached viable cells that had remained attached. No significant difference in cell survival was noted between cells cultured in WE medium or L15 medium.

Cell morphology. Accompanying the decrease in the number of surviving cells during the first 8 d was a dramatic change in cell morphology. After 2 h attachment in complete L15 medium, 94% of cells secured to the plastic tissue culture flasks were viable (trypan blue dye exclusion).

TABLE 1

EFFECTS OF SERUM CONCENTRATION AND DURATION OF PLATING ON MOUSE LIVER CELL ATTACHMENT[a]

Serum Concentration (%)	Serum Lot[b]	1	2	3	4
0	I	0.03 ± 0.01[c] (0.01 ± 0.01)	0.03 ± 0.01 (0.01 ± 0.01)	0.06 ± 0.01 (0.02 ± 0.01)	0.06 ± 0.02 (0.03 ± 0.01)
	II	0.04 ± 0.01 (0.01 ± 0.01)	0.05 ± 0.01 (0.02 ± 0.01)	0.07 ± 0.02 (0.03 ± 0.01)	0.06 ± 0.01 (0.04 ± 0.02)
5	I	0.26 ± 0.02 (0.02 ± 0.01)	0.32 ± 0.02 (0.04 ± 0.01)	0.31 ± 0.02 (0.06 ± 0.02)	0.32 ± 0.02 (0.10 ± 0.03)
	II	0.29 ± 0.02 (0.20 ± 0.01)	0.34 ± 0.03 (0.30 ± 0.01)	0.33 ± 0.03 (0.07 ± 0.02)	0.32 ± 0.03 (0.09 ± 0.02)
10	I	0.37 ± 0.02 (0.03 ± 0.01)	0.43 ± 0.02 (0.03 ± 0.01)	0.42 ± 0.02 (0.08 ± 0.02)	0.41 ± 0.03 (0.11 ± 0.03)
	II	0.38 ± 0.03 (0.03 ± 0.01)	0.44 ± 0.03 (0.03 ± 0.01)	0.40 ± 0.03 (0.09 ± 0.03)	0.40 ± 0.02 (0.10 ± 0.02)
15	I	0.36 ± 0.03 (0.03 ± 0.01)	0.41 ± 0.02 (0.04 ± 0.01)	0.40 ± 0.02 (0.10 ± 0.03)	0.41 ± 0.02 (0.13 ± 0.03)
	II	0.39 ± 0.02 (0.04 ± 0.01)	0.42 ± 0.02 (0.05 ± 0.01)	0.41 ± 0.03 (0.12 ± 0.03)	0.42 ± 0.03 (0.13 ± 0.03)
20	I	0.37 ± 0.03 (0.04 ± 0.01)	0.40 ± 0.03 (0.09 ± 0.02)	0.42 ± 0.02 (0.11 ± 0.02)	0.41 ± 0.02 (0.15 ± 0.03)
	II	0.38 ± 0.03 (0.04 ± 0.01)	0.42 ± 0.02 (0.08 ± 0.02)	0.43 ± 0.04 (0.12 ± 0.04)	0.40 ± 0.03 (0.12 ± 0.03)

[a] Values represent the mean ± standard error of the mean of 10 experiments in which each variable was made in triplicate. Cells were counted in situ. Medium used was L15 and gentamicin (50 μg/ml).

[b] Two lots of serum (I and II) from two different sources were compared.

[c] Values represent the number of attached viable cells (× 10^6) ± SD/25 mm^2 plastic tissue culture flask into which 1 × 10^6 viable isolated cells were plated with 5 ml medium.

Values in parentheses represent the number of attached nonviable cells (× 10^6) ± SD/flask.

TABLE 2

LIVER CELL SURVIVAL: COMPARISON OF WILLIAMS' WE AND LEIBOVITZ'S L15 MEDIA ON PERCENTAGE OF ATTACHED VIABLE AND NONVIABLE MOUSE HEPATOCYTES DURING CULTURE[a]

Medium	Time in Culture					
	2 h	1 d	2 d	4 d	6 d	8 d
Williams' WE	100	88.2	72.4	59.3	35.4	30.6
	(100)[b]	(182.4)	(273.5)	(244.1)	(138.2)	(85.3)
Leibowitz's L15	100	88.3	65.6	53.3	40.7	29.1
	(100)	(163.9)	(261.1)	(255.6)	(113.9)	(88.9)

[a] Values represent the mean of the percentage of originally attached (2 h) viable cells (trypan blue excluding) that remained attached during the culture period. Initially 1×10^6 viable cells were plated on to 25 cm^2 plastic tissue culture flasks. Medium was supplemented with 10% heat inactivated fetal bovine serum and antibiotics. Values represent the percentage of viable hepatocytes of the 1×10^6 initially plated viable hepatocytes in five experiments using triplicate flasks for each value (15 data points).

[b] Values in parentheses represent the mean of the percentage of originally attached nonviable cells (trypan blue staining) that remained attached during the culture period.

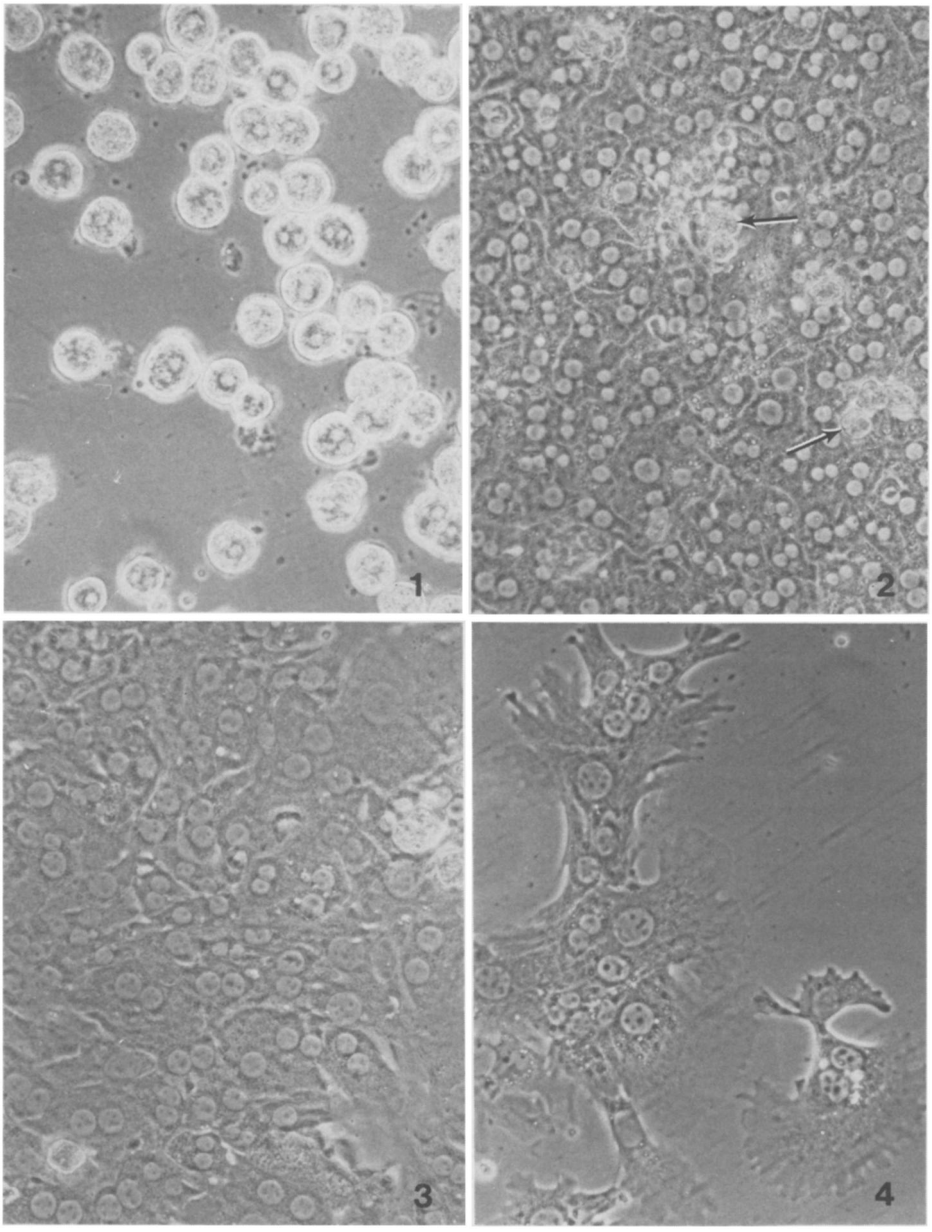
Viable cells appeared spherical under phase contrast microscopy. Often cells were found in small groups of up to 10 cells. These cells formed a single layer (one cell in height) on the plastic substrate (Fig. 1). Approximately 40% of the attached cells appeared isolated, as single cells. Nonviable (trypan blue staining) cells were observed both in association with viable cells in small groups and as individual cells. Thus the complexing of cells into small groups did not appear to influence the cell viability. Many attached cells were binucleated (over 85%). This percentage resembled that seen with the freshly isolated cells. When viewed on cross section, the initially attached hepatocytes still retained a somewhat spherical shape but were flattened on the edge that communicated with the substrate. Some cellular debris also was attached to the substrate. After 2 h attachment, the amount of debris was significant; however, the amount increased proportionately with the increase in duration of attachment.

After 1 d culture in complete L15 medium, all viable hepatocytes appeared flattened on the substrate. The high percentage of binucleated cells (still over 80%) was more apparent after 1 d due to the flattened appearance. Over 95% of the cells examined displayed a morphology consistent with that of hepatocytes. The cytoplasm of these cells was highly granular. The majority of cells now appeared in contact with neighboring cells. This appeared to be the result of flattening and merging of previously individual cells. Cells could be classified into three categories depending upon their association with other cells. Small groups of 5 to 50 cells were very common. Larger groups of hundreds to thousands of cells also were observed (Fig. 2). The latter were usually limited to less than 20 groups per flask. Both groups were found in one or two configurations; sometimes appearing as long cords one to three cells wide although in other cases they were found in somewhat ovoid formations. Individual cells were the third type of configuration observed. These usually represented approximately 15% of the total attached viable cell population.

Cell borders of hepatocytes after 1 d culture generally were rounded although an occasional hepatocyte displayed one or two long cytoplasmic projections onto the substrate. These projections were only exhibited by individual cells or cells on the outer border of a group of cells. Hepatocytes found within the groups usually were four to six sided. The area of cell-to-cell contact appeared linear between adjacent membranes. Rounded profiles of cellular material appeared above the plane of the attached cells but remained in contact with the substrate. This material was stained with trypan blue and was thought to represent cells that had died during the 1st d culture. Nonviable cells were also present within the attached cell population and were distinguishable from the viable cells only by trypan blue staining.

After 2 d culture, a widespread, generally diffused decrease in cell number was noted in both large and small groups of hepatocytes. Some dramatic changes in cell morphology also were readily apparent. Numerous projections of the cellular cytoplasm onto the substrate were evident in most of the attached individual cells. Cell-to-cell junctions were for the most part represented by a clear, linear region between the membranes

(Fig. 3). Cells found on the outer border of the groups also contained cytoplasmic projections onto the substrate (Fig. 4). Cells within the groups that surrounded an acellular area also had projections. These cytoplasmic projections, therefore, were seen whenever cell-to-cell contact was



FIGS. 1-4. Phase contast light micrographs of mouse liver cells during primary culture. All magnifications are ×200.

FIG. 1. Mouse hepatocytes after 2 h culture. Cells are attached to plastic tissue culture flasks.

FIG. 2. A central portion of a large monolayer group of hepatocytes after 1 d culture. The majority of cells are binucleated. Several dead cells are apparent projecting from the monolayer (*arrows*).

FIG. 3. The center area of a large monolayer group of hepatocytes after 2 d in culture is shown.

FIG. 4. A portion of a small group of hepatocytes after 2 d culture. Cells along the outer border of the group show numerous cytoplasmic projections onto the substrate.

not maintained. All cells, those within the groups, on the border of a group, and those found singly, displayed a more elongated cytoplasm than was seen after 1 d culture. The nuclei of cells were unchanged and the percentage of binucleated cells was still in the range of 80% of the total cells counted.

After 4 d culture, a general increase in the cellular changes seen after 2 d was apparent. Most cells appeared elongated. Many also displayed multiple cytoplasmic projections onto the substrate. These projections were most obvious between small groups (up to 25 cells) connected by thin bridges of cytoplasm between cells in each group.

These connections appeared to be the result of degeneration of a larger group into several smaller groups where the remaining contacts were represented by elongation of several cells. In the central portion of larger groups of cells, hepatocytes maintained a morphology similar to that seen after 2 d culture (Fig. 5). The majority of cells within the large groups also maintained their binucleate character. Some nuclear changes were apparent, however, in the other hepatocytes. These cells displayed enlarged and elongated single nuclei whereas others appeared multinucleated containing up to four nuclei per cell. The percentage of attached viable cells remaining binucleated was approximately 75%.

After 6 d elongations and multiple projections of the cytoplasm of most cells were even more pronounced than earlier times of culture. Approximately 15% of attached cells were multinucleated (more than two) and over 70% remained binucleated. Other nuclear changes included enlargement and elongation of the nuclei. Nuclear elongation usually paralleled the elongation of cell cytoplasm. Large cell groups remained but showed signs of severe cell depletion. The remaining cells within the central area of the groups usually were binucleated and somewhat oval in form. Nuclei of these latter cells remained spherical and their cytoplasmic granularity also was maintained. Necrotic cells were seen throughout the culture and many remained connected to viable cells on the substrate (Fig. 6).

After 8 d culture the remaining attached cells displayed many of the same morphologic changes observed after 6 d (Fig. 7). In some flasks, however, several islands of small proliferating cells were observed occasionally. These cells were mononuclear with a nuclear-to-cytoplasmic ratio approaching unity (Fig. 8).

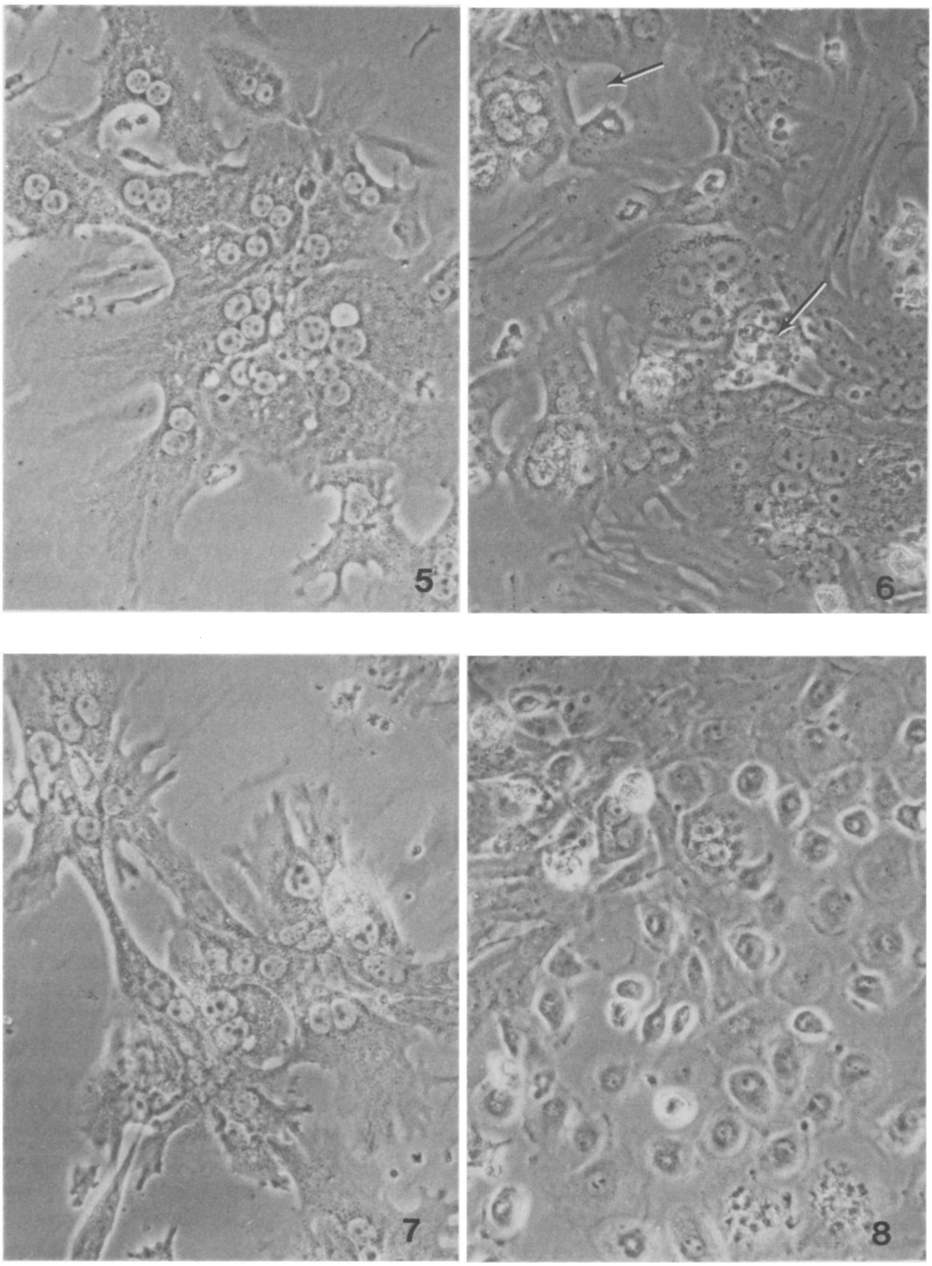
DISCUSSION

This study describes the first reported quantitative investigation into some of the variables influencing attachment and survival of mouse liver cells in primary culture. In general, behavior of cultured mouse liver cells in this study resembled that previously reported for rat liver cells (1-4,6).

The ultimate goal of the attachment experiments was to develop a procedure that would provide maximum viable hepatocyte attachment using the lowest possible concentration of serum in the medium. For attachment of mouse hepatocytes to occur, serum was required in the culture medium. A similar requirement has been reported with rat liver cells (6,7). In the present study, using 10% serum in the medium, maximum attachment of initially plated viable liver cells (43%) occurred after 2 h. When serum concentrations above 10% were used, no significant increase in attachment of viable cells was seen. These findings are in agreement with similar studies on isolated rat hepatocytes in which decreased numbers of attached cells were seen when serum concentrations above 10% were used (3). Conversely, lowering the serum concentration to 5% caused a corresponding decrease in the percentage (33%) of attached viable mouse hepatocytes.

Increasing duration of attachment past 2 h or increasing serum concentration above 10% resulted in greater numbers of attached nonviable (trypan blue staining) hepatocytes. Laishes and Williams (3) noted a similar response with rat liver cells in which a substantial increase (greater than sevenfold) of nonviable but attached hepatocytes were found when attachment duration was over 1 h. With mouse hepatocytes, this increase was approximately twofold after the 2 h attachment period. This difference may be due in part to the percentage of nonviable cells in the initial cell isolate. In the rat (3), viability of the isolated cells was less than 50% whereas the mouse liver cell isolate in this study consistently contained greater than 90% viable cells. The lower cell viability of the plated isolated rat hepatocytes could have contributed to the increased numbers of attached but nonviable cells.

Based on experimental evidence (data not shown) we consider it unlikely that the increased numbers of attached nonviable cells that occurred with increased attachment times resulted from the death of previously attached viable cells or from the expenditure of essential medium component(s). When cells were allowed to attach for



FIGS. 5–8. Phase contrast light micrographs of mouse liver cells during primary culture. All magnifications are ×200.

FIG. 5. A small group of hepatocytes after 4 d culture. Cells are elongated in shape with numerous cytoplasmic projections apparent on the substrate.

FIG. 6. Central area of a large group of hepatocytes following 6 d culture. Cells are elongate in shape. Extracellular spaces between hepatocytes from cell attrition are apparent (*arrows*).

FIG. 7. Remnants of a large monolayer group of hepatocytes after 8 d culture. The large group appears to be broken up into smaller groups through cell attrition.

FIG. 8. After 8 d culture, colonies of small monolayer cells are sometimes apparent in the flask. These cells display a morphology that contrasts with that seen with hepatocytes (*upper left hand corner*). These monolayer cells resemble liver epithelial cell lines morphologically.

1 h and then refed with fresh medium, no change in the absolute numbers of attached viable or nonviable hepatocytes was seen (even after 4 h). Similarly, "conditioned" medium from cultures already allowed to attach for 4 h had no influence on absolute numbers of viable or nonviable cells. If, after 3 or 4 h, the medium lacked or exhausted an essential constituent needed to maintain cell viability, the "conditioned" medium fed to the 1-h old cultures would presumably affect attached cells in a manner similar to that seen after 4 h. Such was not the case. In a similar manner, viable cells dying during the first few hours of culture would have been detected in both the replenished fresh medium and the conditioned medium. Thus, it seems that the cell isolate and not the previously attached cells is the source of additional nonviable cell attachment. Increasing serum concentration had an as yet undetermined promoting influence on attachment of these cells with increased incubation time. This point needs further investigation.

Renton et al. (19) reported an attachment efficiency of over 70% after plating isolated mouse liver cells onto floating collagen gels. Although this is considerably higher than the optimal attachment we noted (43%), the data from the Renton et al. report (19) are difficult to assess inasmuch as no method for determination of attachment efficiency was given.

Similar variations in attachment efficiency of isolated rat liver cells under identical culture conditions have been reported by different groups (2,3,5,6). These differences have been attributed to the method used for determining the number of attached cells. We believe that the in situ counting method used in this study and by others (2,6) is superior to other techniques for determining the number of cells attached to culture substrate. No physical or enzymatic removal of attached cells is necessary to facilitate the countings. Also, as has been shown in this study and that of Williams et al. (3,6), a significant number of the attached cells stained with trypan blue. Chemical methods used by others for counting the number of attached cells, such as DNA and protein determination, do not differentiate the nonviable from the viable attached cells.

Hepatocyte survival and morphology during the first 8 d after attachment mimicked the trend noted previously for adult rat liver cells in culture. However, unlike the rapid loss of attached viable cells reported by several investigators (1-3,7,11) after 1 d culture in the rat hepatocyte system, the mouse liver cells displayed a more gradual decrease in survival in this earlier time period. After a 2 h attachment, viable cells exhibited an approximate 20% daily loss until Day 4. A 40% decrease in viable cells was noted after the 4th to 6th d.

Cell survival decreased only slightly from the 6th to the 8th d culture. The number of attached viable hepatocytes after the 8th d remained fairly constant at approximately 10% of the initially plated viable cells for up to 3 wk culture (data not presented).

The progression of morphologic changes observed in mouse liver cells during culture resembled those reported previously for rat liver cells (2,3,11). Immediately after attachment and for the first 2 d culture, hepatocytes appeared as flattened, polygonal cells. From the 2nd d culture on, cells become more elongated. Further changes in cell morphology depended in part upon whether cells were in groups and therefore in contact with other cells. In general, hepatocytes in apposition to other hepatocytes, was a prerequisite for maintenance of the polygonal shape. This became readily apparent when, through cell attrition, the groups began to undergo dissolution. When cells lost contact with neighboring cells they sent cytoplasmic processes onto the plastic substrate. Cells that remained within the center of the larger monolayer groups and maintained cell-to-cell contact on all sides retained a polygonal pattern and cytoplasmic morphology; even after 8 d that was characteristic of the first 2 d culture. Further investigation into the requirements of maintaining the monolayer groups is needed. Hormonal additions to the culture medium in the primary rat liver cell culture system have been shown to prolong survival of the cells in primary culture (11). Increased survival was accompanied by a prolonged maintenance of the flattened polygonal shape of the hepatocytes. Similar studies of medium additions are needed with primary cultures of mouse hepatocytes to evaluate possible survival and prolonged morphological characteristics.

The hepatocyte characteristics of the mouse liver cell cultures in this study were a prominent, easily recognized morphologic feature. The hepatocyte is the only cell type in the liver that is binucleated to a great extent. Over 85% of the cells placed in culture were binucleated. This proportion decreased during the culture period but still remained at over 70% after 6 d. Nonparenchymal liver cells such as fibroblasts and sinu-

soidal lining cells were seen occasionally, but represented a minor finding.

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